CHEMICAL CHANGES IN BEECH LITTER UNDER MICROBIOLOGICAL DECOMPOSITION*

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THE decomposition of plant materials has hitherto been studied extensively, and the loss of the individual constituents in its course was followed in detail by WAKSMAN, TENNEY and STEVENS (1928), NORMAN (1929), SHRINKHANDE (1933) and many others; but much more work is required on the process of decomposition under natural conditions with reference to micro-flora participating in it.

In the previous paper (SAITÔ, 1956) were described the microbial population and its activities in beech litter. The present paper deals with the chemical composition of beech litter in varied stages of decomposition and the relationships between chemical and microbial changes in it.

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MATERIALS AND METHODS

The materials were taken on August 2, 1956, from the same beechwood in the vicinity of the Tsuta hot spring (Aomori Prefecture) as described in the previous paper, with the exception of fresh leaves which were collected in October of last year in the neighbourhood of the Sukayu hot spring**.

The vertical section through the so-called L- and F-layers in the beechwood may be subdivided into I- to V-layers (*see* SAITÔ, 1956), in which the following types of leaves in varying degrees of decomposition are distinguished:

1. *Fresh leaves*: dead leaves which have just fallen on the ground in the autumn.
2. *Brown leaves*: the vast majority of the brown leaves practically not yet subject to microbial attack lie in I-layer.
3. *Yellowish leaves*: the change in colour from brown to yellow is prominent in the early stage of decomposition. The yellowish leaves are found in II-layer mixed with the next thin yellowish leaves.
4. *Thin yellowish leaves*: the decomposition advances more than the above yel-

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lowish leaves in these. These are found together with the yellowish leaves in II-layer.

5. *Mouldy leaves*: they are found in III-layer, and are prominently yellowish and characterised by an overgrowth of basidiomycetous mycelia.

6. *Fibrous leaves*: they show a residual appearance to some degree and are distributed in IV- and V-layers mixed with the following grayish brown leaves.

7. *Grayish brown leaves*: these are found in IV- and V-layers with the fibrous leaves. It is of interest that they seem not to have been subject to a remarkable decomposition.

As the decomposition does not always proceed uniformly on the whole surface of a leaf, the parts in an aberrant state of decomposition were cut off in advance of analysis for the uniformity of the sample.

The following groups of plant substances were determined by the modified SCHORGER'S method: (1) ash, (2) cold-water soluble substances, (3) hot-water soluble substances, (4) alcohol-benzene (1:2) soluble substances, (5) pentosans, determined by the standard method of boiling with 12% hydrochloric acid and precipitating furfuraldehyde with phloroglucin, (6) cellulose, determined by a modification of the chlorination method of CROSS and BEVAN, (7) lignin, not acted upon by 72% sulphuric acid after a mild hydrolysis, the lignin figure being obtained by subtracting the ash, (8) total nitrogen, estimated by the usual KJELDAHL method.

CHANGES IN THE CONSTITUENTS OF LITTER DURING DECOMPOSITION

MELIN (1930), recently TSUTSUMI (1956) and others reported that there is no definite correlation between the chemical composition of fresh leaves and the rate of decomposition. Of the decomposition of beech leaves in particular, MELIN (1930) mentioned that the comparatively low nitrogen content may be one of the causes of their slow decomposition. There is a widespread opinion that the chemical changes in the organic materials under natural conditions seem to depend on the types of microbes involved as well as on the environmental conditions. Moreover, the activities of small animals are also said to participate in the degradation of litter.

Table 1 shows the proximate composition of leaves in varying stages of decomposition. In analysing the results, it must be kept in mind that, when other constituents have been decomposed to a larger extent, the percentage of a certain substance may rise, although the actual quantity has diminished. The rate of the weight of a leaf decomposed to a certain extent to that of the original brown leaf may be roughly estimated from the coefficient of weight—the dry weight in gram per square decimeter of the area of a leaf as described in the previous paper. It is approximately 85% in yellowish, 74% in thin yellowish, 64% in mouldy and 60%

Table 1. Proximate composition of leaves in varied stages of decomposition, as given on the dry basis.

	Ash	Cold-water soluble substances	Hot-water soluble substances	Alcohol-benzene soluble substances	Pentosans	Cellulose	Lignin	Total-N
Fresh leaf	6.22	7.87	15.65	8.22	17.59	31.70	34.26	0.62
Brown leaf	7.64	3.99	14.41	5.89	17.50	30.79	35.44	0.82
Yellowish leaf	8.98	15.33	20.81	4.95	17.04	31.42	20.68	1.01
Thin yellowish leaf	9.51	13.42	22.28	5.51	15.54	30.87	20.51	1.24
Mouldy leaf	11.09	24.20	33.04	7.33	12.71	27.21	16.87	1.99
Fibrous leaf	12.26	6.43	13.08	5.34	12.62	39.43	17.11	1.43
Grayish brown leaf	8.87	6.04	13.98	4.03	10.94	23.30	44.85	1.69

in the fibrous leaves. The "actual amounts" of individual constituents present in every type of leaf in every stage of decomposition are given in Table 2, where those in the original brown leaf are reckoned to be 100.

Table 2. Changes in the actual amounts of the constituents of decomposing leaves, as given on the basis of the brown leaf.

	Ash	Cold-water soluble substances	Hot-water soluble substances	Alcohol-benzene soluble substances	Pentosans	Cellulose	Lignin	Total-N
Brown leaf	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Yellowish leaf	99	325	125	85	82	86	64	101
Thin yellowish leaf	92	248	114	69	65	71	52	108
Mouldy leaf	93	386	148	80	46	57	30	152
Fibrous leaf	97	97	58	55	43	77	29	102

Cold- and hot-water soluble substances

The reduction of cold-water soluble substances in the brown leaf as compared with the fresh leaf (Table 1) is probably due to the leaching by rain and snow. As will be see from Table 2, the actual amounts of both cold- and hot-water soluble fractions increase, on the whole, with the progress of decomposition. Thus, the largest amount of cold-water soluble substances four times as much as the original amount is to be found in the mouldy leaf. While, from the studies by WAKSMAN and GERRETSEN (1931) and others, water soluble substances are known to be the

first to be decomposed, microorganisms may transform insoluble substances into soluble forms at the beginning of decomposition, leading to an increase in the latter. In the fibrous leaf, however, more or less diminutions are noticed in both water soluble fractions. And, the contents of these fractions in the grayish brown leaf are much the same as in the former (Table 1).

Alcohol-benzene soluble substances

It is said that alcohol-benzene soluble substances retard decomposition, while, on the other hand, there is a conflicting view that they disappear rapidly. In this analytical result, they decompose rather rapidly at least in the early stages of decomposition.

Pentosans

According to WAKSMAN, TENNEY and STEVENS (1928), pentosans are decomposed rapidly, possibly more rapidly than any other group of water-insoluble substances in fresh material. KLEBERG (1927) reported, that the content of pentosans in the organic layers of forest soils decreases with depth, and that they are decomposed first slowly and later rapidly. In reality, both the content and actual amount of them are reduced rapidly as decomposition proceeds.

Cellulose

It is known that, under favourable conditions, cellulose tends to disappear rapidly and does not occur in a large amount in much decomposed material. In this case, however, the rate of decomposition of cellulose is slower than that of lignin except for the grayish brown leaf. The explanation for this phenomenon may be found in that the chemical transformation of plant materials during their decomposition varies with the types of microorganisms involved. In this connection, the fact that the cellulose content even in the grayish brown leaf, which seems to have been subject to no considerable decomposition, is only about 23%, shows that cellulose disintegrates to some extent. This is also accounted for by the ease with which it may be broken to pieces by hand.

Lignin

It is generally accepted that lignin is the most resistant constituent of plant materials. WAKSMAN, TENNEY and STEVENS (1928) and TSUTSUMI (1956) suggested that the presence of lignin is not directly detrimental to the decomposition of plant materials, but disturbs it as the result of a protective coating of cellulose. In their chemical studies on the decomposition of lignin in decaying beech wood, HIGUCHI and the KAWAMURAS (1955) reported that conifenyl aldehyde, vanillin and syringaldehyde may be formed from lignin as degradation products. It is to be noted that

some basidiomycetes, so-called white-rotting fungi, are capable of breaking down lignin with a greater rapidity than cellulose.

In this study, the disappearance of lignin is striking, and this is admittedly caused by basidiomycetes whose mycelia are found on yellowish, thin yellowish and especially mouldy leaves. Thus, the amount of lignin left in the mouldy leaf after the vigorous growth of basidiomycetous mycelia is about 30 % of that in the original brown leaf. Although WAKSMAN and GERRETSEN (1931) have reported even an actual increase in lignin, especially at low temperatures, no increase in lignin is recorded here either in the content or in the actual amount. In marked contrast with the types of leaves infected with basidiomycetes, the grayish brown leaf practically without their attack does show a very high content of lignin.

Total nitrogen

The content of nitrogen is known to be often a limiting factor in determining the rate and extent of decomposition. As will be seen from Tables 1 and 2, total nitrogen increases both in the content and in the actual amount with the progress of decomposition and reaches the maximum in the mouldy leaf. As regards the transformation of nitrogen in plant materials, there are detailed accounts by WAKSMAN and TENNEY (1927), WAKSMAN and GERRETSEN (1931), RICHARDS and NORMAN (1931) and others.

THE GROWTH OF FUNGAL MYCELIA IN INOCULATED LEAVES

To throw light on the growth of fungi in the beech litter, an attempt was made to cultivate basidiomycetous mycelia and four species of imperfect fungi both isolated from the beech litter itself on a series of decomposing leaves moistened with the washing of air-dry litter. Thus, a definite amount of unsterilised leaves were inoculated with bits of leaves infected with basidiomycetous mycelia on one hand, and with a spore suspension of each of the filamentous fungi on the other. The

Table 3. Fungal growth on inoculated leaves in varied stages of decomposition.

	Brown leaf	Yellowish leaf	Mouldy leaf	Fibrous leaf	Grayish brown leaf
Basidiomycetous mycelia	+++	++	+		+++
Absidia glauca	+	++	+++	++	+
Trichoderma viride	++	+++	++++	+++	+
Penicillium lapidosum	+	++	+++	++	+
Penicillium herquei	+	++	+++	++	+

quantitative estimation was made after 5 days' incubation at 22°C. of the basidiomycetous mycelia and after 3 days' incubation at 26°C. of the filamentous fungi.

The results are given in Table 3. The mycelia of basidiomycetes show a better growth in brown and grayish brown leaves, but are poorly developed in mouldy leaves which have already been subject to their attack in the natural habitat. On the contrary, the growth of the filamentous fungi is greatest in mouldy leaves, and is proportional on the whole to the amounts of water soluble substances and total nitrogen. In both brown and grayish brown leaves the development of the filamentous fungi as well as that of the basidiomycetes are much the same. As the leaves used here are not sterile and moreover contaminated with a variety of microbes derived from the litter washing, growths of *Trichoderma* and occasionally of Mucorales are conspicuous with the progress of incubation, and at the same time enormous numbers of various bacteria and protozoa are noticed under a microscope. The development of these contaminants is parallel with the growth of the inoculated filamentous fungi.

To sum up, better conditions for the growth of the filamentous fungi may be created by the previous attack of the basidiomycetous mycelia capable of digesting lignin, the grayish brown leaves got rid of their attack being not so liable to be decomposed by the filamentous fungi.

Relationships between the process of decomposition and the microbial population

Microbial analysis and carbon dioxide production of beech litter have been published in the earlier paper.

As far as the decomposition of litter in this beech stand is concerned, basidiomycetes, likely lignin-decomposing white-rotting fungi, seem chiefly responsible for the initial decomposition of brown leaves. Thus, the ratio of lignin and cellulose (L/C) varies gradually from 1.14 in the original brown leaves to 0.66 in yellowish and thin yellowish, 0.62 in mouldy and 0.43 in fibrous leaves. In reality, three toadstools, two of which were of *Collybia* spp. and one was of *Mycena* sp., were found near the places where samples were taken, and densely moulded yellowish leaves undergoing intense decomposition were noticed around the fruiting bodies.

According to Falck (1926), different types of fungi are concerned in the decomposition of cellulose and lignin. Namely, *Merulius*, *Coniophora* and *Poria* decompose cellulose rapidly, lignin being largely left intact, and the reverse is true of *Polyporus annosus*. Campbell (1932) divides the white rot fungi into three groups based on the differences in biochemical activities as follows: (1) early attack on lignin and pentosans; (2) early disintegration of cellulose and its associated pentosans, and delayed

attack on lignin; and (3) early decomposition of both lignin and cellulose in varying proportions.

As soon as an increase in water soluble substances and total nitrogen takes place as the result of the decomposition and synthesis by lignin-decomposing basidiomycetes, there occurs a marked rise in the numbers of various groups of microbes. The mouldy leaves in III-layer really harbour the largest numbers of microbes apart from filamentous fungi. This depression of filamentous fungi in III-layer under natural conditions is opposed to the foregoing fact that their best growth is recorded in the mouldy leaves in the inoculation test. The reason for it is not clear. Filamentous fungi, such as *Absidia*, *Mucor*, *Trichoderma* and *Penicillium*, though present in small numbers, no doubt play an active part after the development of basidiomycetes. Generally speaking, in a successive series of leaves from yellowish to fibrous undergoing relatively rapid decomposition, basidiomycetes may be mainly responsible for the initial stages of decomposition, and bacteria, actinomycetes, fungi, etc. are of importance in the subsequent process of decomposition.

The frail grayish brown leaves without attack of the lignin decomposers are characterised by a high ratio of L/C as much as 1.92. In IV- and V-layers consisting of the grayish brown leaves and the fibrous ones, the decrease in actinomycetes is not conspicuous in contrast with the depression of bacteria and fungi in V-layer in particular. According to WAKSMAN and GERRETSEN (1931), a great number of actinomycetes appear at the end of the decomposition of compost. Moreover, WAKSMAN and DIEHM (1931) found that actinomycetes are more active than fungi in the decomposition of cellulose. These informations may be in accord with the fact that actinomycetes are abundant in IV- and V-layers. Nevertheless what kinds of microbes really contribute to decomposing the grayish brown leaves is unknown so far. It is noticeable that they are resistant to decomposition, notwithstanding their relatively high nitrogen content.

SUMMARY

1. Beech litter consists of a series of brown, yellowish, thin yellowish, mouldy (overgrown by basidiomycetous mycelia) and fibrous leaves in increasing degrees of decomposition.

2. Basidiomycetes capable of decomposing lignin are likely to be chiefly responsible for the early stages of decomposition. Thereby an increase in water soluble substances and total nitrogen takes place, leading to a vigorous development of a variety of microorganisms which participate in the subsequent process of decomposition.

3. This is supported by a laboratory experiment with the foregoing various

types of decomposing leaves inoculated with basidiomycetous mycelia or filamentous fungi from the litter itself.

4. Not much decomposed grayish brown leaves often met with in the lower layers of litter have got rid of basidiomycetous attack and are rich in lignin. These, as well as the uppermost brown ones practically not yet decomposed, are, in the inoculation test, liable to be decomposed by basidiomycetous mycelia but resistant at least to the attack of other fungi tested.

5. The alcohol-benzene soluble fraction and pentosans are reduced with the progress of decomposition. Apart from grayish brown leaves, the rate of decomposition of cellulose is slower than that of lignin.

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